Simplified Alcoholic Extraction Procedure for Ammonia in Meat Tissue

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A simplified alcoholic extraction procedure for isolating ammonia and volatile amines from tissue was developed; the procedure requires little sample preparation prior to analysis. Ammonia content of extracts was then determined enzymatically with glutamate dehydrogenase and ammonia plus volatile amines with an ammonia-selective electrode. The coefficient of variation was 2.5 and 2.0% for the enzyme and electrode methods, respectively. The apparent total volatile amine content, defined as the difference between the assays obtained by these two methods, was determined for fresh beef and chicken, processed ham, and frozen shrimp. Of the samples tested, processed ham contained the highest concentration of ammonia plus volatile amines, and shrimp had the highest ratio percentage of volatile amines to ammonia.

Ammonia concentration varies little between different muscles or different carcasses and has been suggested as an indicator of microbial spoilage (Gerhart and Quang, 1979) and aging (Brooks and Ammerman, 1978) of meat. The determination of ammonia in meat tissue, however, is not straightforward, and different methods have been used with varying results.

Classically, Kjeldahl distillation methods have been used to determine ammonia in biological samples. The methods are based on the conversion of ammonium ions to ammonia, which is trapped in acid of known concentration and then measured by titration or photometry. Other techniques used to determine the ammonia content in beef, brain, and kidney tissue were the ammonia-selective electrode (ASE) (Gerhart and Quang, 1979), enzymatic assay (Gerhart and Quang, 1979; Reichelt et al., 1964), or modified Kjeldahl (Gerhart and Quang, 1979; Brooks and Ammerman, 1978) methods. The enzyme method is specific for ammonia whereas the ASE reportedly responds to both ammonia and volatile amines (e.g., methyl-, ethyl-, dimethyl-, diethyl-, and butylamines). Large variations have been reported between ammonia values determined by the Kjeldahl method and either the ASE or enzymatic procedures. The higher values obtained by the Kjeldahl method were attributed to cleavage of amino groups in the protein (Gerhart and Quang, 1979; Reichelt et al., 1964). At alkaline pH, there may be liberation of amines as well as formation of ammonia by deamination reactions (Henry, 1964).

The enzyme and electrode methods require removal of protein from the tissue extract before ammonia analysis. Protein is normally removed by acid precipitation, which often is a tedious, time-consuming procedure that can result in ammonia loss from tissue samples (Gerhart and Quang, 1979). To eliminate this problem, a simplified alcoholic extraction procedure which requires little sample preparation prior to analysis by either ASE or enzymatic procedures was developed for isolating ammonia from tissue.

EXPERIMENTAL SECTION

Sample Source. Samples, preground beef, chicken, processed ham, and frozen shrimp, were obtained from local supermarkets and usually analyzed on the day they were obtained.

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Solvent Preparation. Water used in all determinations was deionized and twice distilled, obtained from a Mega-Pure system (Corning, Corning, NY).

Alcoholic Extraction. Ground beef samples were extracted directly and chicken, processed ham, and frozen shrimp were homogenized for 2 min (Polytron homogenizer, Brinkman Instruments, setting no. 4). An extract containing ammonia but not protein was obtained from 10 g of tissue by the following procedure. The tissue sample was placed in a bottle along with 50 mL of water, and the mixture was stirred for 10 min with a magnetic stirrer. Then, 150 mL of methanol was added, and the mixture was stirred for 20 min. The extract was passed through glass wool before analysis by enzyme or ASE methods. These conditions were optimal and based on the extraction of ammonia from ground meat tissue samples both unspiked and spiked with ammonia. The parameters investigated were extraction time and percent methanol of the extract. The ammonia concentration of the tissue was determined on the basis of the final slurry volume (200 mL).

Acid Precipitation. Tissue samples (10 g) were homogenized in 30 mL of 8% (w/v) cold perchloric acid for 2 min (Polytron homogenizer, Brinkman Instruments, setting no. 4), and the homogenates were then centrifuged at 3000 rpm for 10 min at 5 °C. The supernatant was collected and filtered through glass wool to remove lipid containing solids. The filtrate was again centrifuged, and the supernatant was collected. The precipitate was washed twice with water and centrifuged after each washing. Filtrates were combined, and the solution was adjusted to pH 6 with 20% (v/v) KOH and chilled to 5 °C. The precipitate ($KClO_4$) was removed by filtration, and the filtrate diluted to 100 mL with water before analysis by enzyme or ASE methods.

Sample Enrichment. Ammonia was concentrated on a column containing 2 g Dowex 50W-X8 cation exchange resin which had been washed first with water to remove the acid and then with 1 M KCl to remove residual ammonia trapped on the column from the water wash. Water (10 mL) was added to the column, followed by the sample in a known volume. The ammonia from the sample was eluted from the column with 10 mL of 1 M KCl, and the eluate was used directly for analysis.

Ammonia Determination. *Ammonia-Selective Electrode.* Ammonia in extracts was determined by the known addition procedure. An Orion electrode, 95-10 (Orion Research, Inc., Cambridge, MA), was placed in a known volume of extract which had been adjusted to pH 11 with 20% (v/v) KOH, and the change in potential was recorded

Table I. Evaluation of Alcohol Extraction of Ammonia

treatment	enzyme assay: no. of ground[a] beef samples	enzyme assay: found, μmol/g	enzyme assay: CV,[b] %	ASE assay: no. of ground[a] beef samples	ASE assay: found,[b] μmol/g	ASE assay: CV,[c] %
alcohol extraction	3	5.16	1.5	2	5.91	2.0
acid precipitation	3	5.30	1.8	2	5.44	2.0

[a] Assays were not performed on the same portion of beef. [b] Determinations were run in triplicate ($n = 3$). [c] $n = 5$.

Table II. Ammonia Recovery from Beef Tissue Extract

sample[a] plus ammonia	enzyme: total, μmol/g	enzyme: found, μmol/g	enzyme: recovery,[b] %	ASE: total, μmol/g	ASE: found, μmol/g	ASE: recovery,[b] %
1	10.04	10.22	102	11.00	11.04	100
2	15.06	15.67	104	15.94	16.00	100
3	20.08	18.73	93	20.90	19.44	93

[a] The sample contained 5.80 and 6.40 μmol/g endogenous ammonia by enzyme and ASE assays. [b] $n = 5$.

Table III. Comparison of Enzyme, ASE, and Kjeldahl Methods

aged ground[a] beef samples	enzyme,[b] μmol/g	ASE,[b] μmol/g	Kjeldahl,[c] μmol/g
1	14.8	16.3	24.2
2	20.0	21.2	28.1
3	17.2	18.6	26.5

[a] Stored for 5 days at 5 °C. [b] Assay performed on alcohol extract. [c] Assay performed on intact meat samples.

when a known volume of standard NH_4Cl solution was added. The electrode potentials were measured in 0.1-mV units with an Orionanalyzer, Model 801. After addition of the NH_4Cl standard, the concentration ratio of the sample and standard was obtained from the electrode reponse. The ratio was multiplied by the concentration of added standard to determine the concentration of ammonia in the original sample.

Enzyme. The ammonia present in the alcohol-extracted or acid-precipitated samples was determined by a modified procedure based on the enzymatic (glutamate dehydrogenase) conversion of α-ketoglutarate to glutamate in the presence of ammonia in plasma (Sigma Chemical Co., 1980). This reductive amination is accompanied by oxidation of reduced adenine dinucleotide (NADH) to adenine dinucleotide (NAD). The former has a much stronger absorbance at 340 nm, and the decrease in absorbance is proportional to the ammonium ion concentration.

Distillation. The ground beef sample (10 g) was placed in a 600-mL Kjeldahl flask with 200 mL of distilled water, 10 g of carbonate-free MgO, and several drops of Dow Antifoam A. The mixture was distilled, and the distillate (100 mL) was collected in 20 mL of 0.1 N HCl for titration by 0.05 N NaOH with methyl red as the indicator. The procedure was a modification of that used by Brooks and Ammerman (1978).

RESULTS AND DISCUSSION

Every precaution possible was taken to minimize contamination of the sample with ammonia and volatile amines from atmospheric amines, water, solvents, side reactions, etc. They included using freshly distilled water, making 20% (v/v) KOH daily, and removing laboratory amines from the area where the determinations were carried out. Whenever possible, ammonia and volatile amines were determined at those concentrations which are present in tissue.

Alcoholic Extraction. The alcoholic extraction procedure was evaluated by comparing concentrations of ammonia isolated from tissue samples by this procedure with those isolated by the acid precipitation procedure of Gerhart and Quang (1979) (Table I). Good agreement was found between extraction procedures when the samples were assayed by the enzyme procedure. The acid precipitation extracts gave lower results than the alcoholic extracts when assayed by the ASE procedure, however, probably due to losses incurred during sample workup (neutralization and salt removal) prior to analysis by ASE. The alcoholic extraction was also evaluated by recovery of ammonia in tissue to which known amounts of NH_4Cl standards had been added so that the total ammonia concentration was about 2, 3, and 4 times that of the ground beef sample (Table II). Recovery of ammonia from the tissue was quantitative for a single extraction until the total concentration exceeded 20.90 μmol/g; at such concentrations another extraction was required for quantitative recovery. Therefore, samples of unknown ammonia concentration were extracted at least twice.

Ammonia-Selective Electrode (ASE). Maximum precision was obtained for ammonia determination by ASE when the concentration was about 1 mmol/L. The coeff. of variation (CV) for an aqueous ammonia standard solution at this concentration was 0.55% ($n = 5$). At lower concentrations (0.5 and 0.1 mmol/L), the CV increased to 3.0 and 7.3%, respectively. In this study, we found that the ammonia concentration varied between 0.3 and 1.0 mmol/L or 6 and 19 μmol/g of tissue. The preferred method of analyzing the alcoholic extract of tissue by ASE was to add an aqueous standard of known concentration to the extract rather than vice versa. The preferred me-

Table IV. Ammonia (NH_3) and Volatile Amine (Vol-NH_2) Content of Different Tissue

tissue[a] extract	no. of samples	NH_3[b] plus Vol-NH_2, μmol/g	NH_3,[c] μmol/g	Vol-NH_2,[d] μmol/g	Vol-NH_2/NH_3, %
beef	6	5.90	5.42	0.48	8.9
chicken	3	9.88	7.36	2.52	34.2
ham (processed)	3	18.80	11.64	7.16	61.5
shrimp (frozen)	3	5.92	2.94	2.98	101.4

[a] Alcohol extract. [b] Determined by ASE method. CV was less than 3.0% for all determinations. [c] Determined enzymatically. CV less than 2.0%. [d] Determined by difference.

thod requires the electrode's hydrophobic gas-permeable membrane to be in contact with the extract for a longer period than if the extract were added after the standard. Nonaqueous solvents eventually wet the membrane and destroy its gas-permeable nature. We found that up to 15 determinations could be carried out without decrease in electrode performance, providing contact time of the alcohol extract with the electrode's membrane was minimal and the electrode was placed in aqueous solutions between determinations. The CV for tissue extracts containing about 6 μmol/g endogenous ammonia was 2.0% (n = 5). Tissue extracts containing low levels of ammonia were enriched by trapping the ammonia from the extract on a cationic Dowex 50W-X8 column; ammonia was washed from the column with a small volume of 1 M KCl, resulting in near-quantitative recovery.

Enzymatic Determination. Ammonia was specifically detected in tissue extracts with the enzyme glutamate dehydrogenase. Endogeneous enzymes present in the tissue apparently are not extracted and do not interfere with the assay. Methanol present in the extract is diluted with aqueous reagents and does not interfere with the enzyme assay. The CV for extracts containing 10 μmol/g or less ammonia was less than 2.5%.

Distillation. Higher values were obtained when ammonia in aged meat samples was determined by the modified Kjeldahl method (Gerhart and Quang, 1979) than by either the enzyme or ASE method (Table III) on extracts of the same meat. The higher values probably resulted from cleavage of amino groups in the protein as a result of the high temperature and alkaline pH used for the distillation. In addition, the distillate volume of the Kjeldahl reaction was found to affect the ammonia determination. For example, a 75% increase in the distillate volume resulted in a 52% increase in the ammonia value.

Apparent Total Volatile Amine Content. Subtracting the values obtained by enzymatic assay from those obtained by ASE gave values for the apparent total volatile amine content of the sample. The ratio of total volatile amine content to ammonia content for six ground beef samples varied between 5 and 12% and averaged 8.1% (Table IV). Of the food investigated, processed ham had the highest concentration of ammonia plus volatile amines and shrimp the highest ratio of volatile amines to ammonia (101.4%). The volatile amine values are approximate since the electrode response to volatile amines is not the same as its response to ammonia. For example, the electrode response determined by standard addition for several volatile amines (methyl-, ethyl-, dimethyl-, diethyl-, and butylamine) present in beef ranged between 0.3 and 3.5 times the response to ammonia. However, these same volatile amines did not interfere in the enzymatic assay of ammonia.

These results demonstrate that ammonia can easily be extracted quantitatively from ground tissue with aqueous methanol. This procedure is more rapid and less tedious than the acid precipitation procedure commonly used. Also, for samples containing low concentrations of ammonia, a cation-exchange resin can be used to concentrate ammonia in the alcohol extract, with no loss of precision in the subsequent ammonia determination. The enzyme assay is more accurate than the ASE method for determining ammonia in tissue extracts, since volatile amines do not interfere. The enzyme assay does not require high pH and elevated temperatures, which may lead to high ammonia values by the Kjeldahl method. Such high values result from cleavage of amino groups in protein in the meat samples during the Kjeldahl distillation, and the extent of cleavage varies with pH and distilate volume. The ASE responds both to ammonia and volatile amines. Since the electrode's response to volatile amines is not the same as its response to ammonia, only the apparent total volatile amine content of the extract can be obtained by comparing the enzyme and ASE methods.

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